

Purified Vero cell rabies vaccine and human diploid cell strain vaccine: comparison of neutralizing antibody responses to post-exposure regimens

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(Received 13 September 1985; accepted 2 December 1985)

SUMMARY

Neutralizing antibody responses to conventional rabies post-exposure regimens of human diploid cell strain vaccine (HDCSV) and the new purified Vero cell rabies vaccine (PVRV) were compared in 58 healthy Thai veterinary students. The geometric mean titres (GMTs) of the group given HDCSV were slightly higher than those given PVRV, but on day 28 the peak GMTs of the two groups were statistically similar. The early antibody response to PVRV was unaffected by the addition of passive immunization, whereas the level of HDCSV response was reduced on day 14, so that there was no difference on that day between the GMTs of the two vaccine groups given HRIG. However, by day 91 the GMT of those given PVRV and HRIG was lower than in those given HDCSV alone or with HRIG. The appearance of antibody was less rapid than was observed in previous studies using multiple-site intradermal vaccination. Side effects were trivial. Our results confirm the promise of this new, potentially more economical tissue culture vaccine, but they suggest that the regimen could be improved.

INTRODUCTION

Rabies vaccines of nervous tissue origin, still most widely used throughout the tropical rabies endemic area, are potentially harmful (Vejjajiva, 1967; Lopez Adaros & Held, 1971) and sometimes fail to prevent rabies (Vibulbandhitkij, 1980). Human diploid cell strain vaccine (HDCSV), the first of the tissue culture vaccines for human use, was licensed in 1974 and has proved to be a most effective vaccine

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(Plotkin, 1980). However, its high cost has confined its use to a privileged few in developing countries. Economical regimens employing multiple-site intradermal inoculation could greatly reduce the expense of this vaccine (Warrell *et al.* 1983, 1984, 1985), but it seems unlikely that its production could be increased to satisfy global demand. Recently, doubt has been cast on the potency of some batches of HDCSV (Lemon *et al.* 1984; Centers for Disease Control, 1985) and hypersensitivity reactions have been reported in 1:900 vaccinees in the United States (Centers for Disease Control, 1984).

Several other tissue culture grown vaccines are available (Roumiantzeff *et al.* 1984) but often only within the country of manufacture, and they have the same high cost disadvantage as HDCSV. Clearly, further improvements are needed in the purification and economy of mass production of tissue culture vaccines if allergic complications are to be reduced and these vaccines are to replace nervous tissue vaccines, for example among the three million people given rabies post-exposure prophylaxis each year in India (Singh, 1980).

Purified Vero cell rabies vaccine (PVRV) was developed recently employing techniques new to human rabies vaccine production (Roumiantzeff *et al.* 1984). The use of a rapidly replicating continuous cell line grown on micro-carrier particles in tanks lends itself to potential large scale production and thus to a cheaper vaccine (Fournier *et al.* 1985).

Preliminary clinical tests of pre- and post-exposure regimens with PVRV have demonstrated neutralizing antibody responses equivalent to or more rapid than those reported in earlier studies with HDCSV (Fournier *et al.* 1985; Svjetličić *et al.* 1985; Chaldi *et al.* 1985).

We have, for the first time, made a direct comparison of the rabies neutralizing antibody response to post-exposure regimens of the two cell culture derived vaccines (HDCSV and PVRV) and have assessed the effect of simultaneous passive immunization with human rabies immune globulin (HRIG).

MATERIALS AND METHODS

Thai veterinary students of Kasetsart University, Bangkok who needed pre-exposure prophylaxis against rabies because of their chosen profession were offered vaccination provided they were healthy and had not been vaccinated against rabies in the past. Sixty students were randomly allocated (15 to a group) to the following four post-exposure regimens, all of which follow the manufacturers' recommendations: *Group 1* was given Mérieux PVRV intramuscularly (i.m.) 0.5 ml was injected into the deltoid on days 0, 3, 7, 14, 28 and 91. *Group 2* was given Mérieux HDCSV, 1.0 ml injected i.m. into the deltoid also on days 0, 3, 7, 14, 28 and 91. *Group 3* was treated with PVRV as in group 1 but in addition 20 i.u./kg HRIG (Mérieux Imogram Lot XO 730, 185 i.u./ml) was given i.m. into the gluteal muscles on day 0. *Group 4* was treated with HDCSV as group 2 but was also injected with 20 i.u./kg HRIG on day 0, as for group 3.

The PVRV used was batch S1401 of potency 4.5 i.u./dose by the NIH test. The HDCSV used was batches XO 968 and YO 071 of potency 4.5 and 5.5 i.u./dose by the NIH test.

The immunogenicity of the vaccine was assessed by monitoring the development of rabies neutralizing antibody in blood samples taken on days 0, 7, 14, 28 and

Table 1. Ranges of rabies neutralizing antibody in i.u.

Group no.	Regimen	Day 0	Day 7	Day 14	Day 28	Day 91
1*	PVRV	< 0.2	< 0.2-0.3	1.2-5.8	2.4-5.1	1.2-1.4
2	HDCSV	< 0.2	< 0.2-0.5	9.0-6.8	11-7.2	2.6-4.9
3	PVRV+HRIG	< 0.2	< 0.2-0.3	3.0-3.0	5.2-4.1	1.6-8.1
4*	HDCSV+HRIG	< 0.2	< 0.2	1.0-4.9	5.6-7.2	3.4-2.6

* 15 students per group except groups 1 and 4 with 14.

91, using the rapid immunofluorescent focus inhibition test (RIFFIT) (Smith, Yager & Baer, 1973). This was performed in Lyons on coded sera and the results expressed in international units per ml (i.u.). The sensitivity of the test was 0.2 i.u. and, for purposes of calculation, results < 0.2 i.u. were counted as 0.02. The geometric mean titre (GMT) was calculated for each group and comparisons of \log_{10} values made using Student's *t* test.

Evidence of possible local and systemic side effects of the vaccines and HRIG were sought on each occasion the students were seen. The study was approved by the Ethical Committee of the Faculty of Tropical Medicine, Mahidol University.

RESULTS

Two students with high antibody titres were rejected from the study because one was found to have had rabies vaccine previously and the second demonstrated a typical secondary response and subsequently admitted to having been bitten by dogs many times as a young child. The remaining 58 students who volunteered for the study were aged 18-24 (mean 20) years. Their heights ranged from 139-184 cm (mean 165 cm) and their weight 40-61 kg (mean 53.8 kg). Students in the four treatment groups were comparable in age, size and male:female ratio, and they all received vaccine and gave blood samples on the correct days.

On day 7, antibody was detected in three subjects, all in different groups (Table 1). From day 14 onwards at least 1.0 i.u. of antibody was found in every sample.

Serological response to vaccine alone (Groups 1 and 2)

The GMTs produced by PVRV or HDCSV alone were statistically similar on day 28 when the peak titres of 14.9 and 23.7 i.u. respectively were achieved (Fig. 1). The values for the HDCSV group were, however, consistently higher, and on days 14 and 91 the GMTs were significantly higher than for the PVRV group ($P = 0.05$ and 0.008 respectively). On day 91 the GMTs were 7.3 i.u. for HDCSV and 3.4 i.u. for the PVRV group.

Serological response to vaccines with HRIG

The administration of HRIG with PVRV made no significant difference to the GMTs of the antibody response on any occasion. In contrast, the GMT of the group given HDCSV with HRIG was 7.7 i.u. on day 14, significantly lower than the 19.1 i.u. of the group given vaccine alone ($P = 0.006$). The GMTs of the two vaccine groups given HRIG were, however, similar on day 14.

On day 91, among those given HRIG, the GMT of the PVRV group was significantly lower than that of the HDCSV group ($P = 0.01$).

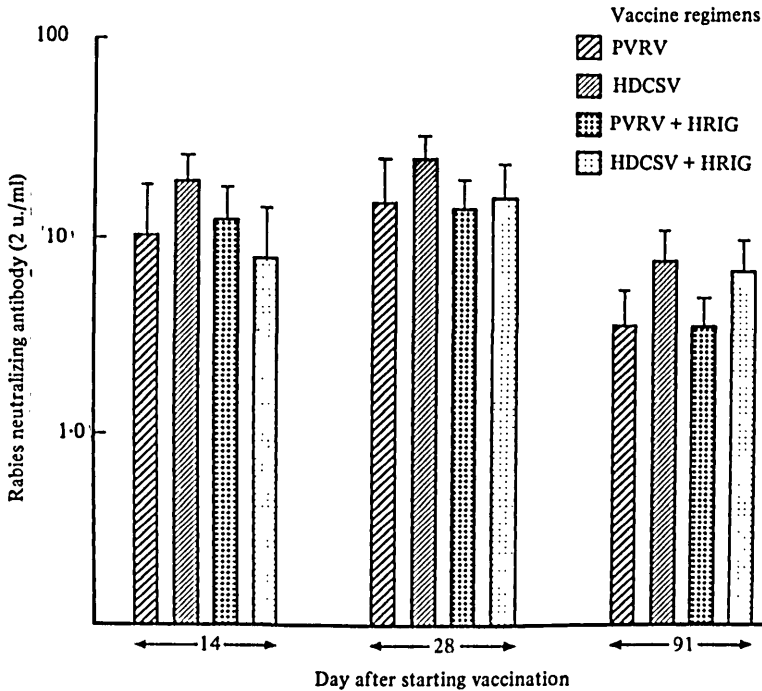


Fig. 1. Geometric mean titres (with 95% confidence limits) of antibody response to vaccines.

Side effects of treatment

The signs and symptoms possibly associated with vaccine treatment were found with equal frequency among students given HDCSV and PVRV (Table 2). Although symptoms were reported by about half the vaccinees, the only abnormal sign detected was axillary lymphadenopathy detected in five subjects.

Pain at the site of HRIG injection lasting 1 or 2 days was reported by nine subjects (four combined with PVRV and five with HDCSV). No other features were associated more often with HRIG than vaccine treatment alone.

DISCUSSION

The neutralizing antibody response is the best available indicator that a vaccine is protective against rabies, but the level of antibody needed for protection is unknown. The aim of post-exposure treatment is therefore to produce a large amount of antibody as fast as possible. In this study, PVRV induced 100% seroconversion with the same speed as HDCSV and with a comparable peak GMT on day 28. The HDCSV GMTs were, however, greater than those for PVRV on every occasion and the difference was significant on days 14 and 91. The side effects of PVRV were trivial, similar in all respects to those of HDCSV.

The serological response to a post-exposure course of PVRV has recently been reported by two other groups. In a study by Svjetlić *et al.* (1985), the peak GMT of 26 i.u. occurred on day 14, in contrast to our peak of 14.9 i.u. on day 28. Otherwise their results were similar to ours.

Table 2. Incidence of symptoms and signs associated with vaccine treatment

Vaccine	Vaccine			
	PVRV		HDCSV	
Group number...	1	3*	2	4*
No. of subjects...	14	15	15	14
Pain at vaccine injection site	3	6	3	5
Pain at HRIG injection site	—	4	—	5
Headache	2	3	2	3
Weakness	2	5	2	2
Felt feverish	1	1	1	2
'Flu-like'	0	0	0	2
Itching at vaccine injection site	0	1	1	0
Dizzy	0	0	1	0
Diarrhoea	1	0	0	0
Axillary lymphadenopathy	2	1	1	1
Symptom free	8	6	10	7
No signs	12	14	14	13
No signs or symptoms	7	6	10	7
Symptom free total	14 (48)†		17 (59)	
No signs total	26 (90)		27 (93)	
No signs or symptoms total	13 (45)		17 (59)	

There is no statistical difference between the two vaccine groups.

* Groups given HRIG.

† Per cent.

From day 14 onwards, the RIFFIT results of Chaldi *et al.* (1985) agree closely with those presented here, but on day 7, 16% of his 75 patients had levels > 0.5 i.u., whereas in our study only one subject given PVRV alone (Group 1) was positive. There was also a single positive in the HDCSV alone group (Group 2). In a study of multisite intradermal vaccination (Warrell *et al.* 1985), HDCSV produced antibody (≥ 0.2 i.u. for comparability with this study) on day 7 in 67% of 42 patients given vaccine alone and 58% of 36 given equine antirabies serum in addition (unpublished data). It may be that multisite PVRV given intradermally may also induce antibody more rapidly, which might be important for patients deprived of passive immunization.

Despite the potency of tissue culture vaccines, antibody is not detectable in the first week of immunization, which could leave a vulnerable period during which rabies might invade the nervous system. Passive immunization is recommended to fill this hiatus (WHO, 1984). A few patients have died of rabies when HDCSV was given without immune serum, even when vaccine was started on the day of the bite (Centers for Disease Control, 1982) or in two cases vaccinated 2 days after being bitten on one or both wrists (Thongcharoen & Wasi, 1985). The manufacturers estimate that this type of failure occurs in one out of 30000–50000 vaccination courses. Nevertheless, immune serum is expensive and inaccessible to the majority of bitten patients, so that acceleration of the response to vaccine by any cheaper means may be life saving.

Significant suppression of the antibody response by HRIG was seen in the HDCSV group on day 14, whereas the GMT following PVRV with HRIG was an unexpected 2.0 i.u. higher than that after PVRV alone. A previous study (Warrell *et al.* 1983) of i.m. HDCSV combined with twice the dose of HRIG, 40 i.u./kg, showed no suppression of the GMT on day 14 so perhaps these results indicate variation, not only between batches of the vaccine and the origin of the serum, but also of the vaccinees.

Three months after starting PVRV treatment with HRIG, the GMT of antibody was significantly lower than that for HDCSV with or without HRIG. Longer term studies of the fall in titre should be carried out in larger groups of subjects before considering abandoning the day 91 booster dose of PVRV as has been suggested for HDCSV (WHO, 1984).

This study demonstrates that the new, more economical PVRV is comparable in all important respects with HDCSV, the most successful vaccine yet developed for rabies post-exposure treatment and the reference vaccine designated by WHO (1984). The addition of passive immunization in the form of HRIG had no suppressive effect on the antibody response. The protective effect of PVRV should now be tested in patients exposed to street rabies virus.

We thank Emeritus Professor Khunying Tranakhit Harinasuta and Professor Danai Bunnag of the Hospital for Tropical Diseases and the staff of the Faculty of Veterinary Medicine, Kasetsart University, Bangkok for their support and assistance; nurses Miss Achara Supapochana, Miss Siripun Arcesuksa and Miss Wattana Theeravuth, and technician Mrs Vanaporn Wuthiekanun for their help; and the students for their co-operation. This study was part of the Wellcome-Mahidol University, Oxford Tropical Medicine Research Programme, funded by the Wellcome Trust of Great Britain.

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